ATTORNEY DOCKET No. 56273/71758 (CPA)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Stinson, *et al*	Art Unit:	1645
Serial No.:	09/215,163	Examiner:	R. Zeman
Filed:	December 18, 1998		
Title:	Humanized Monoclonal Antibodies That Protect Against Shiga Toxin-Induced Disease		

Assistant Commissioner for Patents
Washington, DC 20231

DECLARATION OF HING C. WONG
UNDER 37 C.F.R. §1.132

Dear Sir:

Considered 2/13/03

1. I, Hing C. Wong, declare and say that I am a resident of the United States. My residence address is 2966 Wentworth, Weston, Florida.

2. I hold a Ph.D. degree, which I received from the University of Massachusetts at Amherst in 1980. I currently hold the position of Chairman & Chief Scientific Officer of Sunol Molecular Corporation and President & Chief Executive Officer of Altor BioScience Corp., both of Miramar, Florida. I am an expert in the fields of biochemistry, molecular biology and immunology. My *curriculum vitae* is attached, and illustrates my expertise and experience in these areas.

3. I am a co-inventor of claims 1, 2, 13-20, 23, 29 and 32-41 in the above-identified continuing prosecution application (CPA). I personally conceived, performed and/or assisted in research leading to the claimed invention.

4. I read the Office Action dated September 10, 2002 in the application and understand that the USPTO Examiner has rejected the claims on grounds that the claims are obvious in view of Spiers et al. (Canadian J. of Microbiology 37: 650 (1991); "Spiers") or O'Brien (U.S Pat. No. 5,747,272; "O'Brien") taken with Carter et al. (PCT Publication No. WO 94/04679; "Carter").

5. I have also read the Office Action and understand that the Examiner rejected the claims as being obvious in view of Spiers or O'Brien taken with Shitara et al. (U.S Pat. No. 5,866,692; "Shitara").

6. I have been asked to address what a worker in my field of expertise would conclude from the teachings of Spiers or O'Brien taken with Carter or Shitara as cited by the Examiner in the Office Action.

7. It is my opinion that Spiers or O'Brien taken with the Carter or Shitara references does not make obvious to one working in my field how to make and use the claimed humanized monoclonal antibody. More specifically, I believe that the USPTO was not correct when it concluded on pages 9 and 11 of the Office Action that one would know how to make and express the claimed humanized antibodies in view of Spiers or O'Brien taken with the Carter or Shitara references. My reasons are discussed in the following paragraphs.

8. **None of Spiers, O'Brien, Carter and Shitara provide nucleic acid or amino acid sequence information useful for cloning the 13C4 or 11E10 murine antibodies.** As I understand the Office Action at pg. 9 and 11, the USPTO alleged that an amino acid sequence is inherent to the 13C4 and 11E10 murine antibodies and in view of Carter or Shitara one could make and express the claimed humanized monoclonal antibodies. That is not correct. None of the cited references provide any nucleic acid or amino acid sequence information that would allow one to clone the 13C4 or 11E10 murine antibodies. It was my efforts and those of my co-inventors that led to the cloning of the murine 13C4 and 11E10 variable ("V") regions. The cloning was done without the benefit of pre-existing amino acid sequence information or by determination of such information by us prior to the actual cloning of the V regions. These V regions, once cloned, were used to make the cloned antibodies according to the humanization procedures outlined in my patent specification.

9. **The sequence of the 13C4 and 11E10 V regions could not be readily obtained using the Examiner's cloning approach.** As I understand the Office Action

at pg. 9 and 11, the Examiner implied that a cloning approach described by Carter or Shitara could be used to obtain the sequence of the 13C4 and 11E10 V regions. In my opinion, that approach would not readily work.

10. As I understand the Examiner's position from the Office Action, it is alleged that a worker could obtain amino acid sequence from the 13C4 and 11E10 murine antibodies described by O'Brien and use that information to make a degenerate oligonucleotide probe ("degenerate probe"). Presumably, the probes would be used to screen cDNA libraries prepared from each of the O'Brien hybridomas. However, it would be extremely difficult or even impossible to identify the V regions of the antibodies using this approach. My reasons follow.

11. **Use of the cloning approach cited by the Examiner would not allow straightforward cDNA isolation.** The genetic complexity of a cDNA library is substantial, containing potentially more than 60,000 to 250,000 cDNAs in a library. I understand that the genome of most mammalian cells, includes about 250 to 1000 V_H and about 250 V_L region copies and any one of those V regions could be present in the cDNAs that encode the desired 13C4 or 11E10 V regions. Unfortunately, in the absence of clarifying sequence information, a worker would not know which of the V region genes are present among the potential 60,000 to 250,000 cDNAs in the library and would not know how to design an appropriate probe to identify the cDNA clones of interest.

Further, the cDNA libraries made directly from O'Brien's hybridomas were found to contain antibody pseudogenes. The presence of these pseudogenes confounded the isolation of cDNAs encoding the 13C4 or 11E10 V regions. Thus in the absence of clarifying sequence information, there would be no way to use the Examiner's cloning approach to isolate the cDNAs with any reasonable expectation of success.

11. **I and my co-inventors avoided the cloning approach suggested by the Examiner and successfully isolated cDNAs encoding the 13C4 and 11E10 V regions.** In particular, I and my co-inventors recognized that standard cloning

approaches such as that referenced by the Examiner would require considerable effort and expense to determine the amino acid sequences for both antibodies. We were dissuaded from using the approach to isolate cDNAs encoding the 13C4 and 11E10 V regions for those and other reasons.

12. **A novel combination of oligonucleotides ("oligo cocktail") was used to isolate the 13C4 antibody V region.** My co-inventors and I found that it was possible to isolate cDNA encoding the 13C4 antibody V regions by using a cloning procedure not suggested or taught by the references cited by the Examiner.

13. More specifically, we made an oligonucleotide primer cocktail to isolate 13C4 V region cDNAs from a cDNA library also made by us. The cocktail is disclosed, for instance, in Example 1 of my patent specification at pages 11-12 (bridging paragraph). The cocktail includes a combination of six light chain oligonucleotides (SEQ ID Nos. 7-12) and a corresponding combination of seven heavy chain degenerate oligonucleotide primers (SEQ ID Nos. 1-6 and 15). The **13 distinct degenerate oligonucleotide primers** were developed by us and are not provided or suggested by the references cited by the Examiner.

14. Additionally, Figure 2 of my patent specification shows, among other things, that each of the six heavy chain oligonucleotides we made carries pre-determined codon degeneracy. The exception is the 13C4 specific oligonucleotide ("OKA143"). In view of the intended degeneracy, the heavy chain oligonucleotide cocktail includes **359 discreet sequences** for isolating the 13C4 cDNA heavy chain V region. The particular combination of 359 different oligonucleotides made by me and my co-inventors is not disclosed or provided by the references cited by the Examiner. Significantly, the cited references do not provide any information about which codon degeneracy to choose or how to use them to isolate cDNA encoding the desired V regions.

Figure 2 from my specification also shows, among other things, that each of the six light chain primers includes other pre-determined codon degeneracy. In view of the degeneracy, the light chain oligonucleotide primer cocktail includes **200 discreet**

sequences for isolating the 13C4 light chain V region cDNA. The particular combination of 200 distinct oligonucleotides is not disclosed or provided by the cited references. Moreover, the cited references do not provide any information on the choice of codon degeneracy (see Figure 2) or how to use them to isolate the desired cDNA.

15. These cocktails were used in combinations, such that "front" primer cocktails were used in combination with "back" primers. The isolation of the 13C4 heavy chain V region used a front primer cocktail of six degenerate primers and a single degenerate back primer. Considering the degeneracy and that the primers must work in pairs for successful amplification, the equivalent of **2,118 possible pairs of primers** were used to isolate the 13C4 heavy chain V region cDNA. In practice, each combination of front and back primer was tested in pairs (a total of 6 PCR reactions) and the pair yielding a discrete PCR amplification product was determined empirically by me and my co-inventors. To isolate the light chain V region of 13C4, four degenerate front primers were used in combination with two degenerate back primers, representing a possible **4,224 pairs**. In actual practice, a total of 8 PCR reactions were performed to test all possible combinations. None of the references cited by the Examiner provide any information about how to make or use the oligo cocktail.

16. **I and my co-inventors made another oligo cocktail to isolate the 11E10 antibody variable regions.** We found that it was possible to isolate cDNA encoding the murine 11E10 V regions by using a cloning procedure not provided or suggested by the references cited by the Examiner.

In particular, my patent specification describes this degenerate oligonucleotide primer cocktail. See e.g., Example 4 at pages 19-20. The cocktails include a combination of six specific light chain primers (SEQ ID Nos. 7-12) and eight corresponding heavy chain primers (SEQ ID Nos 1-5, and 28-30). I note that the primers used to clone the heavy chain variable region of 11E10 differ from the primers used to clone the heavy chain variable region of 13C4. As I understand the cited references, they do not provide for or suggest how to make or use the oligo cocktails.

17. Further, Figure 5 of my patent specification shows, among other things, that each of the eight heavy chain oligos carries pre-determined codon degeneracy. In view of the degeneracy of each of the oligos, the heavy chain oligo cocktail includes **544 different sequences** for isolating the 11E10 cDNA heavy chain V region. The particular combination of 544 different oligonucleotides is not disclosed or provided by the references cited by the Examiner. Moreover, the cited references do not provide any information about the choice of the codon degeneracy (see Figure 5) or how to use them to isolate cDNA.

Figure 5 of my patent specification also shows, that the same set of light chain primers was used to clone the light chain variable region of 11E10 and 13C4.

18. These cocktails were used in combinations, such that "front" primer cocktails were used in combination with "back" primers. The isolation of the 11E10 heavy chain V region used a front primer cocktail of five degenerate primers and three degenerate back primers. Considering the degeneracy and that the primers must work in pairs for successful amplification , the equivalent of **67,584 possible pairs of primers** were tried in order to isolate the 11E10 heavy chain V region cDNA. In practice, each combination of front and back primer was tested in pairs (a total of 15 PCR reactions) and the pair giving a discrete PCR amplification product was determined empirically by me and my co-inventors.

To isolate the light chain V region of 11E10, four degenerate front primers were used in combination with two degenerate back primers, representing a possible **4,224 pairs**. In actual practice, a total of 8 PCR reactions were performed to test the possible combinations. The pair yielding a discrete PCR amplification product and used to isolate the 11E10 cDNA encoding the V regions was determined empirically by me and my co-inventors. None of the references cited by the Examiner in the Office Action provide any information about how to make or use the oligo cocktail.

19. **Successful isolation of the cDNAs was found to require a balanced complexity of the oligonucleotide cocktail for V region PCR amplification.** The oligo cocktails were designed to amplify about 80% of the antibody variable regions in an antibody gene library, particularly when about 50 pmoles (500 μM)of the cocktail

was used as a reactant per PCR reaction mix. My co-inventors and I found, by experimentation, that we could amplify 80% of these genes provided a balance between two competing factors in the reaction was maintained, namely, complexity of the oligo cocktail and V region PCR amplification efficiency. That is, we found by experimentation that if the complexity of the oligo cocktail was increased inappropriately (e.g., by increasing codon degeneracy or the number of degenerate oligos), a less representative population of variable gene fragments were obtained. We empirically determined that the oligo cocktails described in my specification, especially when used at the disclosed 50 pmoles (500 μM per reaction], provided the amplification of 80% of the possible V regions that might be present in a cDNA library and were sufficient for the isolation of the cDNAs encoding the 13C4 and 11E10 V regions. None of the references cited by the Examiner teach or suggest these problems or the solution found by me and my co-inventors.

20. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Feb. 7 2003
Date

Hing C. Wong

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BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Hing C. Wong	Chairman of the Board and Chief Scientific Officer, Sunol Molecular

EDUCATION/TRAINING (*Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.*)

INSTITUTION AND LOCATION	DEGREE (*if applicable*)	YEAR(s)	FIELD OF STUDY
National Taiwan University	B.S.	1972-1976	Zoology
University of Massachusetts, Amherst	Ph.D.	1976-1980	Microbiology & Immunology
University of Washington	Postdoc.	1980-1983	Microbiology & Immunology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

RESEARCH AND PROFESSIONAL EXPERIENCE:

1983-1985	Scientist-Gene Expression, Cetus Corporation
1985-1989	Senior Scientist-Gene Expression, Cetus Corporation
1990-1992	Director-Microbial Genetics, Cetus Corporation
1992-1996	Director-Biology Skills Center, Baxter/Dade International Inc.
1996-2001	Founder, President and Chief Executive Officer, Sunol Molecular Corporation
2001-	Founder, Chairman of the Board and Chief Scientific Officer, Sunol Molecular Corporation
2002-	Founder, President and Chief Executive Officer, Altor BioScience Corporation

SELECTED RECENT PUBLICATIONS:

1. C. L. Casipit, R. Tal, V. Wittman, P.-A. Chavaillaz, K. Arbuthnott, J. A. Weidanz, J.-A. Jiao, and **Hing C. Wong** (1998) Improving the binding affinity of an antibdoy using molecular modeling and site-directed mutagenesis. *Protein Science* 7:1671-1680.

2. R. Tal, **Hing C. Wong**, R. Calhoon, D. Gelfand, A. L. Fear, G. Volman, R. Mayer, P. Ross, D. Amikam, H. Weinhouse, A. Cohen, S. Sapir, P. Ohana, and M. Benziman (1998) Three cdg operons control cellular turnover of cyclic di-GMP in Acetobacter xylinum: Genetic organization and occurrence of conserved domains in isoenzymes. *J. Bacteriology* **180**: 4416-4425.

3. A. C. Edwards, A. R. Melton-Celsa, K. Arbuthnott, J. R. Stson, C. K. Smith, **Hing C. Wong**, and A. D. O'Brien (1998) Vero cell neutralization and mouse protective efficacy of humanized monoclonal antibodies against *Escherichia coli* toxins stx-1 and stx2, p. 388-392, *In* Escherichia coli 0157:H7 and other shiga toxin-producing E. Coli strains, ed. J. B. Kaper and A. D. O'Brien. ASM Press, Washington, D.C.

4. J. A. Weidanz, K. F. Card, A. Edwards, E. Perstein, and **Hing C. Wong** (1998) Display of functional αβ scT cell receptor molecules on the surface of bacteriophage. *J. Immunological Methods* **221**:59-76.

5. P. R. Rhode, M. Burkhardt, J.-A. Jiao, A. H. Siddiqui, G. P. Huang, and **Hing C. Wong** (1996) Single-chain MHC class II molecules induced T cell activation and apoptosis. *J. Immunology* **157**: 488-4891.

6. J. R. Stinson, V. Wittman, and **Hing C. Wong** (1995) Generation of single-chain antibody fragments by PCR, p. 300-312, *In* PCR Strategies, ed. M. A. Innis, D. H. Gelfand, and J. J. Sninsky. Academic Press, San Diego, CA.

7. M. de Boer, S.-Y. Chang, G. Eichinger, and **Hing C. Wong** (1994) Design and analysis of PCR primers for the amplification and cloning of human immunoglobulin Fab fragments. *Human Antibodies and Hybridomas* **5**: 57-64.

8. V. Wittman, H. C. Lin, and **Hing C. Wong** (1993) Functional domains of the penicillinase repressor of *Bacillus licheniformis*. *J. Bacteriology* **175**: 7383-7390.

SELECTED RECENT US PATENTS AND APPLICATIONS (not including foreign filings):

1. J. Jiao, D. **H. C. Wong** and E. L. Nieves (filed Dec. 2002) Compositions and Methods for Treating Thrombosis (patent application U.S.S.N. 10/310,113).
2. J. Jiao, D. **H. C. Wong**, L. Leupschen and E. L. Nieves (filed Nov. 2002) Antibodies for Inhibiting Blood Coagulation and Methods of Use Thereof (patent application U.S.S.N. 10/293,417).
3. J. A. Weidanz, **H. C. Wong,** K. F. Card, L. A. Sherman and N. Klinman (filed Nov. 2002) Polyspecific Binding Molecules and Uses Thereof (patent application U.S.S.N. 10/287,941).
4. **H. C. Wong**, J. L. Stinson and L. A. Mosquera (filed Aug. 2002) Method of Humanizing Immune System Molecules (patent application U.S.S.N. 10/230,880).
5. L. A. Sherman, K. F. Card, J.A. Weidanz and **H. C. Wong** (filed June 2002) P53 Binding T Cell Receptor Molecules and Uses Thereof (patent application U.S.S.N. 10/163,084).
6. **H. C. Wong,** V. Wittman, J. A. Weidanz, and H. Belmont (filed Dec. 2001) Transgenic Animals comprising a Humanized Immune System (patent application U.S.S.N. 10/024,648).
7. J. Jiao, D. **H. C. Wong**, L. Leupschen and E. L. Nieves (filed Nov. 2001) Antibodies for Inhibiting Blood Coagulation and Methods of Use Thereof (patent application U.S.S.N. 09/990,586).
8. P. A. Rhode, J. Jiao, M. Burkhardt and **H. C. Wong** (issued Oct. 2001) MHC Complexes and Uses Thereof (**U. S. Pat. No. 6,309,645**).
9. J. A. Weidanz, K. F. Card and **H. C. Wong** (filed June 2001) T Cell Receptor Fusions and Conjugates and Methods of Use Thereof (patent application U.S.S.N. 09/874,907).
10. J. Acevedo, J. Jiao, P. Rhode, **H. C. Wong** and M. Burkhardt (issued May 2001) Soluble MHC Complexes and Uses Thereof (**U.S. Pat. No. 6,232,445**).
11. P. A. Rhode, J. Jiao, M. Burkhardt and **H. C. Wong** (filed May 2001) MHC Complexes and Uses Thereof (continuation patent application U.S.S.N. 09/848,164).
12. J. Acevedo, P. Rhode, J.A. Weidanz, **H. C. Wong**, R. Tal, V. Wittman, M. Burkhardt and K. F. Card (filed May 2001) Modulation of T-cell Receptor Interactions (patent application U.S.S.N. 09/859,012).
13. J. Acevedo, J. Jiao, P. Rhode, **H. C. Wong** and M. Burkhardt (filed Jan. 2001) Soluble MHC Complexes and Uses Thereof (patent application U.S.S.N. 09/766,378).
14. C. Casipit, B. Huang, and **H. C. Wong** (issued Oct. 2000) Binding Molecules and Computer-Based Methods of Increasing the Binding Affinity Thereof. (**U.S. Pat. No. 6,127,524**).
15. J. Jiao, D. P. Taylor, **H. C. Wong**, L. Leupschen and E. L. Nieves (filed Oct. 2000) Tissue Factor Antagonists and Methods of Use Thereof (patent application U.S.S.N. 09/698,673).
16. C. Casipit, B. Huang and **H. C. Wong** (filed Mar. 2000) Binding Molecules and Computer-Based Methods of Increasing the Binding Affinity Thereof (patent application U.S.S.N. 09/537,368).
17. J. Jiao, D. **H. C. Wong**, L. Leupschen and E. L. Nieves (issued Nov. 1999) Antibodies for Inhibiting Blood Coagulation and Methods of Use Thereof (**U.S. Pat. No. 5,986,065**).
18. J. A. Weidanz, **H. C. Wong,** K. F. Card, L. A. Sherman and N. Klinman (filed Oct. 1999) Polyspecific Binding Molecules and Uses Thereof (patent application U.S.S.N. 09/422,375 - allowed).
19. J. Jiao, D. P. Taylor, **H. C. Wong**, L. Leupschen and E. L. Nieves (filed Sept. 1999) Pharmaceutically Active Compounds and Methods of Use Thereof (patent application U.S.S.N. 09/406,269).
20. J. Jiao, D. **H. C. Wong**, L. Leupschen and E. L. Nieves (filed Apr. 1999) Antibodies for Inhibiting Blood Coagulation and Methods of Use Thereof (continuation patent application U.S.S.N. 09/293,854).
21. P. A. Rhode, J. Jiao, M. Burkhardt and **H. C. Wong** (issued Feb. 1999) MHC Complexes and Uses Thereof (**U. S. Pat. No. 5,869,270**).

22. A. Melton-Celsa, J. L. Stinson, H.C. Wong, C. K. Schmitt and A. D. O'Brien (filed Dec. 1998) Humanized Antibodies that Protect against Shiga Toxin Induced Diseases (patent application U.S.S.N. 09/215,163).

23. P. A. Rhode, J. Jiao, M. Burkhardt and **H. C. Wong** (filed Apr. 1998) MHC Complexes and Uses Thereof (patent application U.S.S.N. 09/067,615).

24. P. A. Rhode, J. Acevedo, M. Burkhardt, J. Jiao and **H. C. Wong** (filed Oct. 1997) Soluble MHC Complexes and Uses Thereof (patent application U.S.S.N. 08/960,190).

25. J. A. Weidanz, **H. C. Wong,** and K. F. Card (filed Oct. 1997) Soluble Single-Chain T-Cell Receptor Proteins (patent application pending U.S.S.N. 08/943,086).

26. J. A. Weidanz, **H. C. Wong** and K. F. Card (filed Mar. 1997) Fusion Proteins comprising Bacteriophage Coat Protein and a Single-Chain T Cell Receptor (patent application U.S.S.N. 08/813,781).

27. **H. C. Wong**, P.A. Rhode, J. A. Weidanz, S. Grammer, A. C. Edwards, P.-A. Chaviallaz and J. Jiao (filed Jan. 1997) MHC Complexes and Uses Thereof (patent application U.S.S.N. 09/900,379).

28. **H. C. Wong**, P.A. Rhode, and J. A. Weidanz (filed Jan. 1997) MHC Complexes and Uses Thereof (patent application U.S.S.N. 08/776,084).